A COMPARATIVE STUDY OF THE CHROMOSOMES OF LACHNOSTERNA (COLEOPTERA).

E. L. SHAFFER,

DEPARTMENT OF BIOLOGY, PRINCETON UNIVERSITY.

A. INTRODUCTION.

The studies presented here were begun in the spring of 1916 at Princeton University and were continued until the spring of 1917. Most of the material used was collected at Cold Spring Harbor during the summer of 1916.[1] The studies were discontinued in the summer of 1917 owing to the war. On returning to Princeton last spring, it was thought advisable to assemble the observations previously made despite the fact that they did not represent as complete a study as had been intended.

I wish to express my sincere thanks to Professor E. G. Conklin for much valuable assistance and encouragement.

B. MATERIALS AND PLAN OF STUDY.

It was originally intended to make a detailed study of the process of synapsis as well as a comparative study of the chromosomes of four selected species of May beetles, genus *Lachnosterna*. While the material was not entirely favorable for these purposes, some interesting facts were brought to light.

The four scarab beetles of the genus *Lachnosterna* which were selected for study were the species *delata*, *fusca*, *gracilis* and *tristis*. Besides these, for comparative purposes, two other scarab beetles were studied, *Pelidonota punctata* and *Cotalpa lanigera*. The form most studied was *L. delata* and since the other forms showed no essential differences from *delata*, the latter will be used as the basis of description in the present paper.

Comparatively little detailed study of spermatogenesis in the Coleoptera has been done. The work of Miss Stevens ('05, '06),

[1] The writer wishes to express his thanks to the Brooklyn Institute of Arts anp Sciences for the privileges of a research fellowship at the Laboratory of the Institute at Cold Spring Harbor, L. I., during the summer of 1916.

while it covered a large number of species, was concerned only with chromosome counts, and especially with reference to the sex chromosomes. In only two species of beetles has there been anything like a detailed study of the chromosomes in synapsis, Voinov (1903) on *Cybister roselii* and Schäfer (1907) on *Dytiscus marginalis*. Both these authors after a detailed study of the growth stages of the spermatocytes describe parasynapsis, while Miss Stevens claims telosynapsis in the forms she studied.

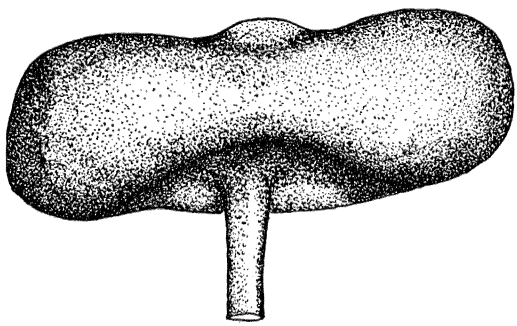


FIG. 1. Single testis of *Lachnosterna* (side-view), with its efferent duct.

The material used in this study was fixed in Flemming's, Hermann's, Bouin's, Gilson's and Carnoy's fixing fluids. In general, the Flemming and the Hermann material was best for the growth stages of the spermatocytes, while the Bouin material was best for the chromosomes. Iron-hæmatoxylin, with and without a counterstain, was employed entirely for staining. Aceto-carmine smears were valuable in checking the observations on the fixed material. All the testes, except those of *L. fusca*, were taken from the adult beetles. The material was gathered in midsummer and showed all stages from spermatogonia to ripe spermatozoa. In the case of *L. fusca*, the adult testes showed few favorable stages and it was necessary to study the larval gonads.

C. DESCRIPTION OF TESTES AND SERIATION OF STAGES.

The testes consist of twelve mushroom-like bodies, three pair in each side of the abdomen. Each testis has its duct (Figs. 1 and 2) and the ducts from each group of testes unite to form two larger ducts; these four larger ducts in turn unite to form the single median vas deferens.

The testes, although of an unusual shape, show the seriation of the stages clearly, being but a modification of the simple straight (orthopteran) type with a linear seriation of the cells. In the species studied, the testes consist of a great many follicles radiating from the center. Fig. 2 represents a diagrammatic section through the center of the testis and perpendicular to its broad surface. In the center of the testis from which the follicles radiate (Fig. 2, *A*), one finds all the spermatogonia and here new cysts are in the process of formation. On each side of this

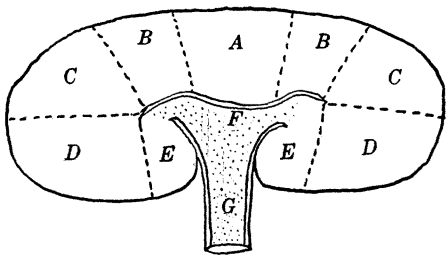


FIG. 2. Diagrammatic section through testis at right angles to its broad surface, to show seriation of stages. *A*, region in which are all spermatogonia; *B*, early growth stages and synezesis; *C*, pacyhtene and later growth stages; *D*, maturation divisions; *E*, spermatids and spermatozoa; *F*, cavity in testis where spermatozoa are retained prior to discharge from testis; *G*, efferent duct.

region (*B*, *B*) are the early synaptic stages (synezesis). In the regions *C*–*C* one finds the later synaptic stages (pachytene and diplotenes), while in regions *D*–*D* show most of the spermatocyte divisions; regions *E*–*E*. contain most of the spermatids and spermatozoa. The chamber (*F*) shown in the figure serves as a place where the spermatozoa are collected and stored prior to discharge from the testis; the duct (*G*) leads from the storage chamber. Of course the stages above seriated overlap and there is no sharp delimitation as is diagrammatically shown in the figure. The formation of the cysts in the region A was followed out and my observations confirm those of Wieman ('10) and Hegner ('14) that each testicular cyst is derived from a single spermatogonium. There is, however, no evidence that cell division is by amitosis as Wieman found.

D. Observations.

1. *Spermatogonia and Diploid Chromosome Groups.*

In all of the species of *Lachnosterna* studied, as well as *Pelidonota* and *Cotalpa*, the diploid number of chromosomes as shown in the spermatogonia is twenty, including an unequal (sex) pair (Figs. 1–6). Dividing follicle cells in the ovaries show ten equal pairs of chromosomes (Fig. 7). There are three pairs of J- or U-shaped chromosomes, one pair of which is considerably larger than the others (Figs. 1–5, *AA*). The sex chromosomes are the smallest in the complex, consisting of a very small round chromosome (*y*) and a somewhat larger rod-shaped chromosome (*x*). In comparing the size relations of the chromosomes in the several species studied, one finds no marked differences. In many cases the chromosomes in the diploid complexes are arranged in pairs, homologous chromosomes lying beside each other. In the Diptera, Metz (1916) has found that pairing of chromosomes is not confined to the maturation stages, but at each cell division homologous chromosomes come together. In the Diptera the diploid chromosome number is relatively low; in species where the chromosome number is high, pairing of homologous chromosomes is usually not found to be so complete. It therefore seems that chromosome pairing, outside of the maturation stages, is related to chromosome number.

In the spermatogonial telophases, the chromosomes spin out into fine chromatic threads (Fig. 13) and as the nucleus grows the threads become more and more complex forming a chromatic reticulum or typical resting nucleus. This "resting" nucleus is of relatively short duration, for soon the chromatin begin to condense into heavier threads (Fig. 15), and as condensation continues, all the chromatin of the nucleus becomes confined into large chromatic blocks of a granular nature (Figs. 16, 17). Counts of these chromatic blocks in uncut nuclei always approximate the diploid chromosome number and these blocks may be considered as the anlages of the future spermatogonial chromosomes. The blocks consist of a linin core on which are imbedded the chromatin granules; they are connected to each other by a fine net-work of linin which seems to be continuous with the linin forming the core of the blocks. Most of the cells in the spermato-

gonial area of the testes are in this stage and apparently it is of of much longer duration than the reticular or "resting" stage.

In some respects the chromatic blocks above described correspond to the "prochromosomes" which have been described by Overton ('09) in *Podophyllum*, Arnold ('08) in *Hydrophilus piceus*, Goodrich ('16) in *Ascaris incurva* and other workers. In these cases, however, the chromatic bodies appeared at the beginning of the growth period and, according to the above workers, these bodies arranged themselves in pairs, thereby accomplishing the synaptic process. In *Lachnosterna* there is no such paired arrangement of the chromatic blocks; they merely represent stages in the formation of the spermatogonial chromosome groups and might really be called prophases, except that they are of relatively long duration. In some cases a precocious longitudinal split can be detected, preparing the chromosome for the next cell division.

2. *The Synaptic Stages and Maturation Divisions.*

Following the telophase of the last spermatogonial division (Fig. 13), the chromosomes spin out in the form of very fine (*leptotene*) threads (Fig. 18, 19) which entirely fill the nucleus and prevent a minute analysis of this stage. The actual pairing of the homologous chromosomes could not be followed in detail, but observations on a few favorable cells (Fig. 19) indicate that the union is side-to-side (*parasynapsis*). Stevens ('06) has described telosynapsis in the Coleoptera, but she did not make a study of the early growth stages.

The leptotene stage gradually merges into a definite contraction stage (synezesis) with all the chromatic threads polarized at one side of the nucleus (Figs. 20, 44). These stages are always found in a definite part of the testes, namely in region B (Fig. 2), and are found nowhere else. McClung ('05) used the word "synezesis" to describe that "condition of the nucleus in which the chromatin is found massed at one side of the vesicle, without regard to whether it is a normal phenomenon or not." McClung and recently some of his students, Whiting ('17) and Hance ('17), have maintained that a unilateral massing of the chromatin or synezesis is an artifact and is due to improper

fixation methods. With this in mind, the writer has sought with most careful technical methods to obtain fixed material which might not show these contraction figures; but without exception cells in the contraction phase were always found in the definite region of the testes mentioned above. It is quite true that poorly fixed material shows an abundance of contraction figures, but in these cases, as will be shown later, they are just as likely to appear in other regions of the testes than in the very definite location above mentioned. There is no doubt that even the best fixation will tend to emphasize the contraction of the chromatin just as it does in the case of the other cell structures, but synezesis is unquestionably a normal process in the beetles studied here. Whiting ('17) has advanced the idea that the chromatic elements during synapsis are in an unstable condition and that "any shock is likely to cause them to clump together." It is questionable whether good fixation is much less of a "shock" than indifferent fixation. It is conceivable that true synezesis may not occur as a normal phase of the maturation possesses in some animals (*e.g.*, Orthoptera), but the fact that it has been described by many workers using a variety of fixing methods supports the fact that it is a normally occurring phase in some cases. In any event it proves that the nuclear condition is peculiar in cases of synezesis.

Following the stage of synezesis, the chromatin threads are released from the polarized bouquet in the form of thick ragged looking *pachytene* threads (Fig. 21). Usually a longitudinal split can be seen in the threads, which marks the point of synapsis of the homologous threads. The chromomeres are imbedded in a linin base, chromomeres of the same size lying opposite each other and being connected with each other by fine linin threads. In the later stages the threads become more widely separated from each other (Fig. 23) assuming the *diplotene* form. In these stages and in still later ones, the threads show a variety of twisting about each other forming rings with and without crossed ends, figures 8, double and even triple crossing-over of the threads. In no case could a secondary split be seen. The *strepsistene* threads continue to become more widely separated and it soon becomes impossible to trace the individual threads (Fig. 22).

This unanalyzable stage is of relatively short duration and is followed by a condensation of the chromatin in the form of heavy threads (Fig. 25). Condensation of the chromatin continues and the definitive maturation tetrads begin to make their appearance. In these early prophases one often finds cells in which all the chromatin is massed at one side of the nucleus (Fig. 26), resembling very much a synezesis figure. Gross ('07) has described a second synezesis in *Pyrrhocoris* and Mottier ('07) believes that in the plants it is a regularly occurring phase in the maturation processes. In *Lachnosterna* these contraction figures are most abundant in material which is poorly fixed and I consider them as artifacts. Fig. 27 represents this stage from well fixed material as contrasted with Fig. 26 from poorly fixed material.

All the first spermatocyte metaphase plates of the four species of *Lachnosterna* studied, as well as *Pelidonota* and *Cotalpa*, show ten bivalent chromosomes the smallest of which represents the sex pair (Figs. 8, 9, 10, 11, 12). These are usually arranged in characteristic groups with fine linin threads connecting the various members of the complex to each other. A comparison of the tetrads of *L. delata* with those of *L. fusca* (Figs. 28, 29) shows no marked differences either in form or in size of the tetrads. Using Miss Carothers' ('17) nomenclature, there are five atelomitic tetrads (non-terminal spindle fiber attachments) and five telomitic tetrads (terminal spindle fiber attachments). The atelomitic tetrads are the largest in the complex and are derived from the three pairs of J-shaped spermatogonial chromosomes and two pairs of the bent rod-shaped ones. In sideview metaphases, the largest of the tetrads (Figs. 28, 29) has a sub-terminal spindle fiber attachment, and is derived from the *AA* pair (Fig. 5) of the diploid chromosome group which also have subterminal fiber attachments. The other atelomitic tetrads consist of two typical crosses and two annular tetrads of the *Stenobothrus* type. The other four autosome tetrads are of the ordinary dumb-bell type while the *x* and *y* elements (sex pair) are fused end to end (Figs. 28, 29).

The types of tetrads above described are found in all four species of *Lachnosterna* studied. On the other hand, in *Cotalpa*

and *Pelidonota* no cross-shaped tetrads and only one ring tetrad are found. The question of reduction division is difficult to analyze here, with the exception of the ring tetrads. The latter are always arranged on the spindle in the direction of the spindle axis and the spindle fiber attachment is median. Consequently the separation of the dyads occurrs at the point of the synaptic union and the division is reductional.

3. *Sex Chromosomes.*

The earliest work on the sex chromosomes was done by Miss Stevens ('05, '06) on the Coleoptera. She found the so-called sex chromosomes in over forty species and her work and that of Wilson's on the Hemiptera and McClung's on the Orthoptera have been the basis of the later work correlating sex determination with the chromosomes. In the Coleoptera the sex chromosomes are found as unpaired "accessory" and as unequal elements which separate in one of the maturation divisions and divide equationally in the other maturation division. Arnold ('08) has maintained that in *Hydrophilus piceus* there are no sex chromosomes. There is present in the growth stages a chromatin nucleolus which may even persist up to the first maturation division and may even pass undivided to one cell. However, it disappears and cannot be found in any of the second spermatocytes.

In *Lachnosterna*, *Pelidonota* and *Cotalpa* the sex chromosomes are of the *xy* type the *y* element being the smaller of the unequal pair (Figs. 1–6). There are no marked differences in the size and form of the sex chromosomes in the four species of *Lachnosterna* studied, but in *Pelidonota* the *x* element is considerably larger than in the *Lachnosterna* material. In all cases the sex pair separate in the first maturation division and divide equationally in the second, thus yielding two types of spermatozoa. (Figs. 31, 32, 33, 35, 36). In a single case the sex chromosomes failed to separate in the first maturation division, both chromosomes going into one of the daughter cells. This is undoubtedly a case of non-disjunction similar to that which has been found genetically and cytologically by Bridges ('16) in *Drosophila*.

The sex chromosomes presist throughout the entire growth

period as definite compact chromatic bodies. They are always contained within a chromosomal vesicle such as has been described by Wilson ('12) in *Oncopeltus* and *Lygæus* (Figs. 21, 25). In *Lachnosterna* the sex elements usually remain separate from each other, each enclosed in a separate vesicle. In *Pelidonota* and *Cotalpa*, the sex pair remain fused during the synaptic period, the smaller (*y*) element usually being imbedded along the side of the larger (*x*) element.

E. General Considerations.

1. *Chromosome Number and Species.*

The intensive work of McClung and his students on one family of Orthoptera, the Acrididæ, has shown that the chromosome number in all the species studied of this group is the same, namely 23 in the male. This has led McClung to the generalization that species closely related taxonomically might show similarity in their chromosome groups. It is very evident that this generalization cannot apply to all groups since, in some cases there is a wide divergence in chromosome number among members of the same genus. It is possible that in some cases this difference in chromosome number between closely related species may be due to a fusion of several chromosomes or else a breaking up of one or more chromosomes into several distinct components. In the case of *Hesperotettix*, McClung ('17) has shown that the chromosome number may vary from 17 to 23. He has shown that these variations are due to a fusion of chromosomes resulting in the formation of "multiple chromosomes." In one species, *Hesperotettix viridis*, he has found the haploid or reduced number to vary from 9 to 13. On the other hand, the work of Stevens on the *Diabrotica*s (Coleoptera) has shown that the species *vitatta* has 21 chromosomes, while the species *soror* and 12-*punctata* have but 19. However, in the latter two species there may be present from 1 to 4 additional or "supernumary" small chromosomes. It is quite possible that the supernumary chromosomes of the species *soror* and 12-*punctata* represent the fragments of a pair of chromosomes, which would therefore make an agreement in chromosome number between these two species and the species *vitatta*. As McClung ('17, p. 545) has pointed

out, he has confined his idea of this similarity of chromosome number in closely related species, only to the family Acrididæ. It is possible that in other forms correspondence in chromosomes may extend only to the subfamily or genus. In the Hemiptera and Coleoptera certainly there is no such uniformity of chromosome number in the various families as is found in the Acrididæ.

The four species of *Lachnosterna* studied here differ from each other very much as far as taxonomic characters are concerned, nevertheless the chromosome groups show no difference either in form or number. The two other forms studied, *Pelidonota* and *Cotalpa*, differing generically, have the same chromosome number (20 in the diploid groups), but there are some differences in the form of the maturation tetrads. Only one other scarab bettle has been studied, *Euphoria inda* by Stevens ('06), and it corresponds with a diploid group of 20 chromosomes, so that all the species of the family *Scarabidæ* thus far studied correspond in chromosome number. The genus *Lachnosterna* embraces over one hundred species, some very much alike so that it is difficult to separate them taxonomically, others differing markedly from each other. The most constant difference is found in the male copulatory organs, which probably prevents the interbreeding of species in nature. Perhaps further cytological studies in this genus will yield results similar to those in the Acrididæ. Certainly there is a wealth of material for such a comparative study.

2. *Cyst Formation and Cell Polarity.*

Hegner ('14) has studied the formation of spermatogonial cysts in the testes of *Leptinotarsa;* the facts concerning cyst formation in the beetles studied here show results essentially similar to those of Hegner. The primary spermatogonia are not arranged in cysts and are more or less polygonal in shape, with the nucleus usually located in the center. Cyst formation begins by the rapid division of a single primary spermatogonium, together with an adjacent epithelial cell which forms a follicular membrane around the cyst. Consequently we can say that all the cells within any one cyst are the descendants of a single primary spermatogonium. With the formation of the cyst,

the spermatogonia are arranged in the form of a rosette, and are now triangular or wedge-shaped with the nucleus at the base and the rest of the cytoplasm extending toward the cyst cavity. Thus, with the formation of the cyst, there is a polarity established in the spermatogonia which is maintained up to the formation of the ripe spermatozoa, for, the side where the nucleus is located is destined to form the head of the spermatozoon, and the cytoplasmic portion extending toward the cyst cavity is destined to form its tail. Hegner ('14) has homologized the process of cyst formation with the differential divisions in insect oogenesis which establish nurse cells and oöcytes. It has long been known that the insect egg possesses a remarkable polarity besides being highly organized. Since, as it has been above shown, the polarity of the sperm cells are established at the time of cyst formation, and since this process is homologous to nurse cell-oöcyte differentiation, it is probable that the polarity of the egg may have its origin at the time of the differentiation of nurse cells and oöcytes.

3. *Linin and Chromosome Structure.*

When one studies the history of the chromatin of the nucleus from the resting stage through the synaptic period up to the reconstitution of the definitive maturation chromosomes, one begins to seek for some of the underlying mechanisms concerned in the movements of the chromatin particles. From the diffuse granular state of the chromatin up until the formation of the chromosomes, the linin network of the nucleus plays an active part. Chromatin granules in the nucleus are never isolated as such, but always have linin connections with other granules. The synaptic threads consist of linin threads with the chromomeres embedded along them. As has been before stated, homologous chromomeres have linin connections running between them. Conklin ('17) has shown that the ground-work of the cytoplasm is the relatively stable and elastic spongioplasm, and he attributes to it the maintenance of cytoplasmic organization and the movements and localization of cytoplasmic substances. Similarly in the nucleus it seems that the linin is a relatively elastic substance which forms the ground-work of the nucleus

and maintains the organization of the nuclear elements. Wenrich ('16) has shown how remarkably constant the organization and "architecture" of the chromosomes are. By means of certain structural peculiarities which his "selected" chromosomes presented, he was able to recognize and trace them through all the stages of spermatogenesis. The tendency has been noted in many forms for the chromosomes to appear in the metaphase always in a definite configuration. It is possible that the linin connections between the chromosomes which have often been figured (Figs. 8–12) are responsible for the definite patterns assumed by the chromosomes in the metaphase plate. The uniting in pairs of the homologous leptotene threads may be due to the contractility of the linin connectives running between the homologous chromomeres. In short, the morphological stability of the nuclear elements and the constancy of their form, arrangement and organization is in the last analysis referable to the linin.

F. Summary.

1. The diploid chromosome groups of four species of *Lachnosterna*, namely *delata*, *fusca*, *gracilis* and *tristis*, as well as *Pelidonota punctata* and *Cotalpa lanigera*, show twenty chromosomes, one pair of which is composed of two unequal elements (sex chromosomes).

2. There are no essential differences in the form and arrangement of the chromosomes in the species studied.

3. The growth period of the spermatocytes is marked by the appearance of delicate leptotene threads which are derived from the chromosomes of the last spermatogonial division. These threads become polarized and there is evidence that they are arranged in pairs parasynaptically.

4. There is a definite contraction stage which does not seem to be caused by fixation, but is a normally occurring phase in the growth period.

5. The sex chromosomes persist through the entire growth-period in the form of definite compact bodies, sometimes being contained within chromosomal vesicles. The unequal sex elements separate in the first maturation division and divide equationally in the second maturation division.

6. There are five atelomitic tetrads in the first maturation division and five telomitic tetrads (including the sex pair).

7. Cyst formation in the testis begins by the rapid division of a single primary spermatogonium, so that all the cells within any particular cyst are the descendants of a single cell. The visible polarity of the cells seems to be established at the time of cyst formation.

G. Addenda.

Since the manuscript of the foregoing study was written, the work of Goldsmith[1] has appeared on the chromosomes of the Cicindelidæ. He has studied in all five species of this family and finds that they agree in chromosome number. He has described a double odd-chromosome which passes undivided to one pole of the spindle in the first maturation division and divides in the second maturation division giving rise to spermatozoa with ten and twelve chromosomes respectively. In his study of the growth stages of the spermatocytes he has been unable to find that the leptotene threads actually pair. His figures of the synaptic stages are not clear and he makes no decision as to the method of synapsis (parasynapsis or telosynapsis).

He describes the "early" spermatogonia as being arranged in syncytia without any discernible cell-walls. He describes the appearance within the syncytial cytoplasm of "cytoplasmic fibrillar bridges." "With the increase in age and size of the cells, these bridges become more dense and assume a definite arrangement about a number of cells. This continues until the entire tubule is subdivided into a large number of syncytia—cysts containing cells without perceptible cell walls" (p. 445). Both his descriptions and figures of this peculiar method of cyst formation lack in clarity. From his Fig. 5, I interpret the "cytoplasmic fibrillar bridges" as being probably the persisting spindle remains or mitosome of the previous division, and it is also probable that the deeply staining bodies in the cytoplasm are the mid-bodies (cell-plate) persisting with the mitosome. That these "fibrillar bridges" are really spindle remains is further

[1] A comparative study of the chromosomes of the tiger beetles (Cicindelidæ). *Jour. Morph.*, Vol. 32, No. 3, 1919.

indicated by the fact that they" become more dense and assume a definite arrangement about a number of cells." This is exactly the behavior of the spindle remains which Hegner[1] has described in Leptinotarsa and which I have described in *Passalus*.[2] It is difficult to see how these "fibrillar bridges" are concerned in dividing the syncytia into a number of cysts. Furthermore, it is difficult to believe that a true syncytium of spermatogonia actually does exist, for the later stages certainly do possess cell-walls which must have been preëxisting.

LITERATURE CITED.

Arnold, G.

'08 The Nucleolus and Michrochromosomes in the Spermatogenesis of *Hydrophilus piceus*. Arch. f. Zellforsch., Bd. 2.

Bridges, C. B.

'16 Non-disjunction as a Proof of the Chromosome Theory of Heredity. Genetics, Vol. 1.

Carothers, E. E.

'17 The Segregation and Recombination of Homologous Chromosomes as Found in Two Genera of Acrididæ (Orthoptera). Jour. Morph., Vol. 28, No. 2.

Conklin, E. G.

'17 Effects of Centrifugal Force on the Structure and Development of the Eggs of Crepidula. Jour. Exp. Zoöl., Vol. 22, No. 2.

Goodrich, H. B.

'16 The Germ Cells in *Ascaris incurva*. Jour. Exp. Zoöl., Vol. 21, No. 1.

Gross, J.

'07 Die spermatogenese von Pyrrhocoris apterus L. Zoöl. Jahrb. Abtheil. f. Anat. u. Ontog., Bd. 23.

Hegner, R. W.

'14 The Germ Cell Cycle in Animals. Macmillans.

Metz, C. W.

'16 Chromosomes Studies on Diptera, II. Jour. Exp. Zoöl., Vol. 21, No. 2.

Mottier, D. M.

'07 The Development of Heterotype Chromosomes in Pollen Mother Cells. Ann. of Bot., Vol. XXI.

McClung, C. E.

'05 The Chromosome Complex of Orthopteran Spermatocytes. BIOL. BULL., Vol. IX., No. 5.

'17 The Multiple Chromosomes of *Hesperotettix* and *Mermiria* (Orthoptera). Jour. Morph., Vol. 29.

Overton, J. B.

'09 On the Organization of the Nuclei in the Pollen Mother Cells of Certain Plants with Especial Reference to the Permanence of the Chromosomes. Arch. f. Mikr. Anat., Bd. 70.

[1] Studies on the Germ Cells, I and II. *Jour. Morph.*, Vol. 25, No. 3, 1914.

[2] Mitochondria and Other Cytoplasmic Structures in the Spermatogenesis of *Passulus cornutus*. BIOL. BULL., Vol. XXII., No. 5, 1917.

Schäfer, F.

'07 Spermatogenese von Dytiscus. Zool. Jahrb. Abtheil. f. Anat. u. Ontog., Bd. 23.

Stevens, N. M.

'06 Studies on Spermatogenesis, II. Carnegie Inst. Pub. No. 36.

'05 Studies in Spermatogenesis with Especial Reference to the Accessory Chromosome. Carnegie Inst. Pub. No. 36.

'08 The chromosomes in *Diabrotica vitatta*, *Diabrotica soror* and *Diabrotica 12-punctata*. Jour. Exp. Zoöl., Vol. V., No. 4.

'09 Further Studies on the Chromosomes of the Coleoptera. Jour. Exp. Zoöl., Vol. VI., No. 1.

Voinov, D.

'03 La spermatogénèse d'été chez le Cybister roeselii. Arch. de Zoöl. Exp. et Gen., 4^{e} Series, T. 1.

Wenrich, D. H.

'16 The Spermatogenesis of *Phyronotettix magnus* with Especial Reference to Synapsis and the Individuality of the Chromosomes. Bull. Mus. Comp. Zoöl., Harvard Coll., Vol. IX., No. 3.

Whiting, P. W.

'17 The Chromosomes of the Common House-mosquito, *Culex pipiens*. Jour. Morph., Vol. 28, No. 2.

Wilson, E. B.

'12 Studies on the Chromosomes, VIII. Jour. Exp. Zoöl., Vol. 13, No. 3.

Wieman, H. L.

'10 A Study in the Germ Cells of *Leptinotarsa signaticollis*. Jour. Morph., Vol. 21.

EXPLANATION OF PLATES.

All drawings (except Figs. 28, 29, 30) were made with camera lucida using No. 12 ocular and 1/16 mm. oil immersion objective. Figs. 28, 29, 30 were made using No. 18 ocular and 1/16 mm. oil objective.

PLATE I.

(Figs. 1 to 12.)

FIGS. 1 to 6. Metaphase plates of spermatogonia in the six beetles studied all showing 20 chromosomes including an unequal pair (*XY*).

FIG. 1. *L. delata.*

FIG. 2. *L. fusca.*

FIG. 3. *L. tristis.*

FIG. 4. *L. gracilis.*

FIG. 5. *Pelidonota.*

FIG. 6. *Cotalpa.*

FIG. 7. Metaphase plate of follicle cell from ovary of *L. delata* showing ten equal pairs.

FIGS. 8 to 12. Metaphase plates of 1st spermatocytes showing ten bivalent chromosomes.

FIG. 8. *L. delata.*

FIG. 9. *L. fusca.*

FIG. 10. *L. gracilis.*

FIG. 11. *Pelidonota.*

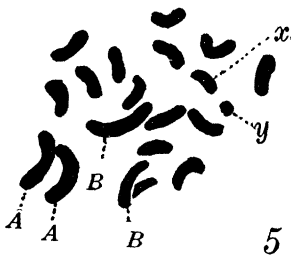
FIG. 12. *Cotalpa.*

1

2

3

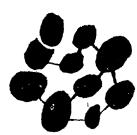
4



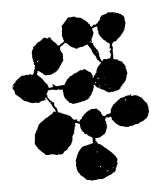
5

6

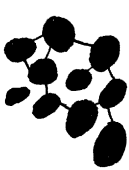
7



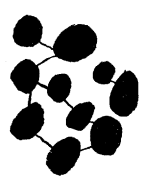
8



9



10



11

12

PLATE II.

(Figs. 13 to 27.)

FIG. 13. Telophase nucleus of spermatogonium, showing chromosomes spinning out into delicate threads.

FIG. 14. Characteristic resting nucleus of spermatogonium.

FIGS. 15, 16, 17. Stages in the condensation of the chromatin from the resting stage to the formation of the chromatic blocks.

FIGS. 18, 19. Early growth stages. Evidences of parallel pairing of leptonete threads.

FIG. 20. Contraction (synezesis) stage.

FIG. 21. Pachytene threads released from synezesis stage.

FIG. 22. Strepsistene nucleus. Chromatin threads unanalyzable.

FIG. 23. Various forms of diplotene and strepsitene threads.

FIG. 25. Early prophase of 1st spermatocyte. Tetrads beginning to form.

FIG. 26. Cell in prophase simulating synezesis; due to faulty fixation.

FIG. 27. Cell in stage similar to Fig. 26 from well-fixed material.

13

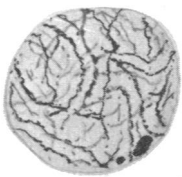
14

15

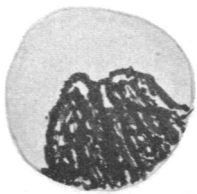
16

17

18



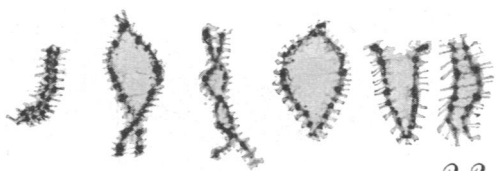
19



20

21

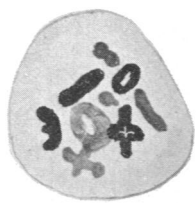
22



23

25

26



27

PLATE III.

(Figs. 28 to 36.)

FIG. 28. Side view of maturation tetrads of *L. delata*.

FIG. 29. Side view of maturation tetrads of *L. fusca*.

FIG. 30. Side view of maturation tetrads of *Pelidonota punctata*.

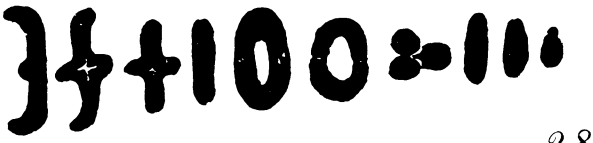
FIG. 31. Early anaphase of first maturation division in *L. fusca* showing separation of sex pair.

FIG. 32. Anaphase of first maturation in *Pelidonota*.

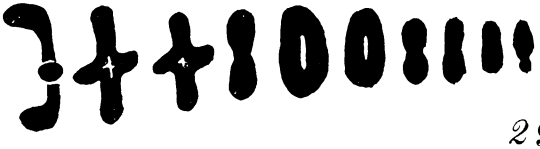
FIG. 33. Telophase of first maturation in *L. delata*.

FIG. 34. Telophase of first maturation in *L. delata* in which the sex elements have failed to disjoin, and have passed to one daughter cell.

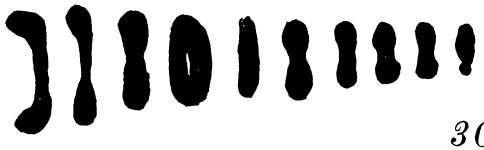
FIGS. 35, 36. Daughter plates of second spermatocyte of *L. delata* and *Pelidonota*, respectively.



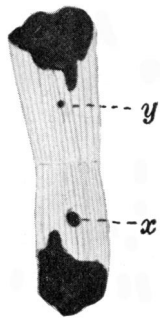
28



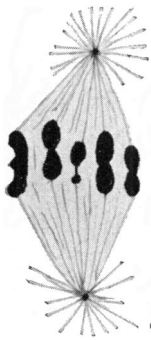
29



30

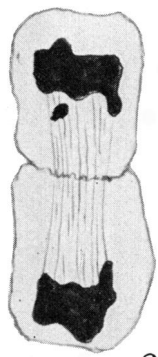


33

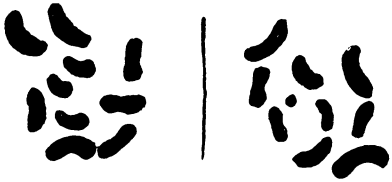


31

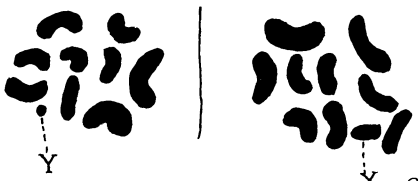
32



34



35



36